

EFFECTS OF SOIL TYPE AND ADULT SIZE ON MATING PROPENSITY AND REPRODUCTIVE OUTPUT IN TWO POPULATIONS OF THE LAND SNAIL *ARIANTA ARBUSTORUM* (LINNAEUS)

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ABSTRACT

Life-history traits in terrestrial gastropods may be influenced by both abiotic and biotic factors. This study examines the effects of soil type and adult size (shell volume) on mating propensity and female and male reproductive output (number and mass of eggs, number of sperm delivered and spermatophore mass) in individuals of the simultaneous hermaphrodite land snail *Arianta arbustorum* from two populations kept both on calcium-(Ca)-rich and Ca-poor soil. Snails from the two populations differed in adult size, relative shell growth, mating propensity and egg size. Furthermore, in both populations the number of egg batches deposited, egg size and spermatophore size scaled allometrically with shell volume, but not the total number of eggs produced and number of sperm delivered. Independent of population and shell size, the type of soil on which the snails were maintained influenced mating propensity, the total number of eggs produced and the mass of the albumen gland (another measure of female reproductive output). The mating propensity was higher and the total number of eggs produced was larger in snails kept on Ca-poor soil than in individuals reared on Ca-rich soil. This surprising finding could be explained by the fact that the Ca-poor soil used in the experiment still contained enough Ca to allow reproduction, and that the snails ingested Ca through the food consumed (lettuce grown on Ca-rich soil was available *ad libitum*). Moreover, the Ca-rich soil could contain minerals or (unknown) substances which discourage reproduction in *A. arbustorum*. Our study highlights the complexities faced when interpreting differences in the life history of gastropods. Explaining interpopulational differences in life-history patterns requires not only the understanding of the influence of snail origin, but also an understanding of the effects of shell size, substratum type (soil type), food and local climate.

Key words: *Arianta arbustorum*, calcium availability, egg size, reproductive allocation, simultaneous hermaphrodite, sperm number.

INTRODUCTION

Life-history theory predicts that a species occurring in different environments exhibits interpopulational variation in life-history traits as a result of different selection pressures (Stearns, 1992). However, observed local differences in life histories may also result from founder effects, genetic drift, and phenotypic plasticity (Calow, 1978; Caswell, 1983). Moreover, evolutionary interpretations of life-history patterns require a distinction to be made between genotypic and environmentally induced phenotypic variation, because life-history variation may also be the result of developmental plasticity or physiological acclimatization. It follows that

observed variation in life history could simply mirror differences in habitat quality.

Reproductive resource allocation is a fundamental aspect of life history with profound ecological and evolutionary consequences (Stearns, 1992). Shelled gastropods strongly depend on calcium (Ca) as a major macronutrient constituent of their body (Dallinger et al., 2001). Besides reinforcing the shell, Ca is critical to a variety of functions in soft-tissue metabolism and reproduction (Tompa & Wilbur, 1977; Porcel et al., 1996). Most of the Ca absorbed by terrestrial gastropods may enter the animal's body via the epithelium of the intestine (Dexheimer, 1963; Beeby & Richmond, 2007). Because of seasonal needs for Ca, snails store

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this mineral mainly as calcium carbonate and possess high reallocation capacities (Fournié & Chétail, 1984). Ca can be mobilized readily from the intracellular storage site during periods of increased demand (Tompá & Wilbur, 1977). Ca provision to the eggs represents a major cost to the parent, and a variety of strategies are used to ensure sufficient Ca for the hatchling to build a shell (Tompá, 1980; Baur, 1994a). It is well established that soil type and calcium availability influence shell growth and female reproductive output (number of eggs laid) in terrestrial gastropods (Baur, 1994a; Heller, 2001). For example, egg production of *Helix pomatia* Linnaeus, 1758, exposed to acid soil was doubled when calcium carbonate was supplied during an experiment (Crowell, 1973). However, the potential effect of soil type on mating behaviour and the male reproductive output (spermatophore size and number of sperm delivered during copulation) have not been investigated in terrestrial gastropods.

Here we present the results of an experiment designed to examine whether mating propensity and sex-specific reproductive allocation in the simultaneous hermaphrodite land snail *Arianta arbustorum* (Linnaeus, 1758) are affected by the type of soil. In a reciprocal transplant experiment, snails from habitats with Ca-rich and Ca-poor soils were kept either on their original soil or on the other soil under laboratory conditions. In particular, we asked whether the origin of the snails, adult shell size and/or soil type influence the mating propensity, number of sperm delivered during copulation, and number of eggs laid within a season in *A. arbustorum*. We also examined whether the ratio of the resources allocated either to the male or female reproductive output is affected by the snails' origin, shell size, and soil type.

MATERIALS AND METHODS

Study Animals

Arianta arbustorum is common in moist habitats of northwestern and central Europe (Kerney & Cameron, 1979). The snail has determinate growth (shell breadth of adults 17–22 mm). Individuals become sexually mature at 2–4 years, and adults live another 3–4 years (maximum 14 years; Baur & Raboud, 1988). In the field, snails deposit one to three egg batches consisting of 20–50 eggs, per reproductive season (Baur & Raboud, 1988; Baur, 1990). In contrast to male fecundity (i.e., sperm

expenditure), female fecundity (i.e., clutch size and the number of batches produced per season) is positively correlated with adult shell size (Baur, 1994a; Baur et al., 1998).

Mating in *A. arbustorum* includes elaborate courtship behaviour with optional dart shooting (i.e., the pushing of a calcareous dart into the mating partner's body), and lasts 2–8 h (Hofmann, 1923; Baur, 1992a). Copulation is reciprocal; after intromission, each snail transfers simultaneously one spermatophore (Haase & Baur, 1995). The spermatophore is formed and filled with sperm during copulation (Hofmann, 1923). It has a distinctive form consisting of a head, a body (sperm container with 800,000–4,000,000 spermatozoa), and a tail 2–3 cm long (Baur et al., 1998). Fertile sperm can be stored for more than one year (Baur, 1988). Mating is random with respect to shell size and different degrees of relatedness (Baur, 1992a; Baur & Baur, 1997). Individuals need at least eight days to replenish their sperm reserves after a successful copulation (Locher & Baur, 1999; Hänggi et al., 2002).

Paternity analysis in broods of wild-caught *A. arbustorum* showed a high frequency of multiple insemination (Baur, 1994b). A controlled laboratory experiment showed that one successful copulation per reproductive season is sufficient to fertilize all the eggs produced by an individual (Chen & Baur, 1993). However, there is a probability of 5–8% that a copulation will not lead to fertilization of eggs (no sperm transfer or transfer of infertile sperm; Chen & Baur, 1993).

Arianta arbustorum feeds on vascular plants and dead or senescent herbs, but the snails often supplement their vegetable diet with microorganisms, soil and carrion (Frömming, 1954; Grime & Blythe, 1969).

General Methods

Subadult *A. arbustorum* were collected from a locality with Ca-rich soil (Gurnigel) and a locality with Ca-poor soil (Zastler) in late April and early May 2002. Snails in the site with Ca-rich soil inhabited the embankment of a track in the subalpine forest near Gurnigel, 30 km south of Bern, Switzerland (46°45'N, 7°27'E; elevation 1,320 m above sea level), those in the site with Ca-poor soil the embankment of a forest road in the Black forest, Germany (47°54'N, 8°01'E; elevation 1,060 m). The two sites were 130 km apart.

Topsoil from both localities was collected as substrata for snail maintenance. Three addi-

TABLE 1. Results of the chemical soil analyses. See text for further explanation of variables.

Soil parameter	Gurnigel, Ca-rich soil	Zastler, Ca-poor soil
Calcium (Ca)*	1,409	99
pH (H ₂ O)	7.2	5.8
Total nitrogen (%)	0.37	0.30
C/N-ratio	11.5	15.2
Phosphorus (P ₂ O ₅)*	0.2	1.3
Potassium (K ₂ O)*	19	17
Magnesium (Mg)*	6	17
Copper (Cu)*	0.03	0.24
Iron (Fe)*	0.6	18.0
Manganese (Mn)*	0.3	9.4
Zinc (Zn)*	0.1	1.7

*in mg/100 g dry soil

tional samples from each site were combined for soil analyses: the percentage of nitrogen (N), the content (in mg per 100 g dry soil) of calcium (Ca), phosphorus (P₂O₅), potassium (K₂O), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), the C/N-ratio and the soil pH in water. The soil samples were analysed using standard protocols by the laboratory of F. M. Balzer, Wetter-Amönau, Germany. Characteristics of the two soils are summarized in Table 1. The Ca content represents both the exchangeable calcium and the calcium carbonate content of the soil.

The snails were kept isolated in transparent beakers (8 cm deep, 6.5 cm in diameter) lined with moist soil (approximately 4 cm) outdoors at a shaded place. Fresh lettuce was provided twice per week and at the same time the beakers were cleaned. Snails from both sites were randomly assigned to one of two treatment groups. Half of the snails were raised on soil from their original locality, the other half on soil from the other locality (Gurnigel: 53 individuals on Ca-rich [original] soil, 52 individuals on Ca-poor soil; Zastler: each 51 individuals on Ca-poor [original] and Ca-rich soil).

Within four weeks, all individuals reached sexual maturity as indicated by the formation of a flanged lip at the shell aperture. The snails were marked individually with letters and numbers written on their shells with a waterproof felt-tipped pen on a spot of correction fluid (Tipp-Ex). The animals showed no visible reaction to the marking procedure.

Shell breadth of each snail was measured twice to the nearest 0.1 mm using vernier callipers immediately after being collected as subadults and after shell growth was completed. Relative shell growth of an individual was expressed by the difference between the two measurements divided by the initial size. In adult snails, shell height was also measured and shell volume was calculated using the formula: shell volume = 0.312 x [(breadth)² x height] – 0.038 (measurements in mm; B. Baur, unpublished data). Shell volume is a more reliable measurement of snail size than weight, because weight depends on the state of hydration and thus is highly variable in terrestrial gastropods.

Sexually mature snails were allowed to mate outdoors. Active snails (individuals with an extended soft body and everted tentacles) from each treatment group were placed in a transparent plastic container, measuring 25 x 18 x 7 cm, lined with moistened paper toweling. When a pair had started to court the snails were transferred to a smaller plastic container (14 x 10 x 7 cm) to allow mating to take place without disturbance from other non-courting snails. Mating trials were initiated in the evening and ran during three nights in June 2002. The period between the end of May and the middle of July is the time of maximum mating activity in subalpine populations of *A. arbustorum* (Baur, 1992a).

We observed the snails' courtship behaviour at intervals of 15 min (at night using a torch) following the method described in Baur (1992a) and recorded courtship duration (time interval from courtship initiation to copulation). Observation sessions were terminated either after successful copulation or after 14 h if no snail initiated courtship behaviour. Snails that did not mate were tested again seven days later with a new composition of snails within the same treatment group. Between two trials, snails of each group were maintained as described above.

After copulation, one randomly chosen mating partner (hereafter referred to as focal snail) was kept in a beaker as in the premating phase, but at 19°C with a light:dark cycle of 16:8 h. The other mating partner (hereafter referred to as sperm receiver) was frozen immediately after copulation. To obtain the spermatophore from the focal snail, we dissected out the female reproductive tract of the receiver. We measured the length (*L*) and width (*W*) of the sperm-containing part of each spermatophore to the nearest 0.1 mm using a dissecting microscope. Spermatophore size (in mm³) was

approximated, by the formula ($\pi LW^2/4$), assuming a cylindrical volume. Spermatophores were kept singly in Eppendorf tubes at -30°C until sperm were counted.

The beakers of focal snails were checked for eggs once per week. The eggs of each batch were collected, counted, and kept in a plastic dish (6.5 cm in diameter) lined with moist paper towelling at 19°C to determine hatching success. Newly hatched snails were separated from remaining unhatched eggs to prevent egg cannibalism (Baur, 1992b). In all treatment groups eggs were collected over a period of 60 days following copulation. The length of this period corresponds to approximately one reproductive season of *A. arbustorum* living in the wild (Baur, 1990).

Sperm Counting Procedure

We assessed the number of sperm that a focal snail delivered to its mating partner by counting the number of sperm in the spermatophore transferred. This procedure is described in detail in Locher & Baur (1997). The spermatophore of *A. arbustorum* consists of a hardened secretion which encapsulates the spermatozoa (Hofmann, 1923). We mechanically disrupted the spermatophore in 200 μl PBS-buffer (138.6 mM NaCl, 2.7 mM KCl, 8.1 mM $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$ and 1.5 mM KH_2PO_4) using a pair of microscissors. The sperm suspension was homogenized with a set of Gilson pipettes for 5–15 min. To count the sperm, we stained the homogenate for 1–3 h with an equal volume of a gallocyanin-chromium complex, which stains the DNA in the head of the spermatozoa. If spermatozoa still occurred in clusters, we treated the sample overnight with a sonicator (35 kHz). Two subsamples of known volume of the sperm suspension were diluted 1:3 with PBS-buffer and transferred to a Bürker-Türk counting chamber. This counting chamber consists of 16 cells each with a volume of 25 nl. We counted all sperm heads in randomly chosen cells until the total number of sperm heads exceeded 400, and used the average of two subsamples to calculate the total number of sperm in a spermatophore.

Estimate of Sex-Specific Reproductive Allocation

As measures of female reproductive allocation the dry mass of all eggs produced by an individual and the dry mass of the albumen gland were considered. We assessed the dry

weight of 48 randomly sampled eggs (three eggs each from eight snails from both populations, equally distributed over the soil types), calculated the mean dry weight of an egg for both populations on each soil type, and multiplied this by the number of eggs produced by each snail. At the end of the experiment, the albumen gland of each focal snail was dissected out of the female reproductive duct. We determined the dry mass of the albumen gland to the nearest 0.1 mg. The dry mass of spermatophores filled with spermatozoa was considered as a measure of male reproductive allocation. We used the relationship between the size of the spermatophores (X = volume of the sperm container in mm^3) and the dry mass of spermatophores filled with spermatozoa (Y in mg)

$$\ln(Y) = 0.670 \times \ln(X) - 0.524$$

to calculate the dry mass of the spermatophores produced by individual snails in these experiments (relationship from Locher & Baur, 2000).

Data Analyses

The StatView program package (SAS Institute, 1998) was used for statistical analyses. Means $\pm$ 1 S.E. are given unless otherwise stated. Data which did not fit normal distributions were $\log_{10}$ -transformed and frequency data (hatching success) were arcsine-transformed. Differences in shell size and relative growth rate between treatment groups (soil type) and populations were examined using two-way analysis of variance. However, allometric relationships may confound interpretations of differences in observed reproductive output between treatment groups and/or populations. To examine possible differences in reproductive traits analysis of covariance with soil type and population as factors and shell size as covariate was used (ANCOVA, type III model).

RESULTS

Shell Growth and Adult Size

At the beginning of the experiment, subadult snails from the Gurnigel population were smaller than those from the Zastler population (shell breadth: 15.0 ± 0.1 mm vs. 17.9 ± 0.1 mm; $F_{1,120} = 324.24$, $p < 0.0001$). However, subadult *A. arbustorum* from either population kept both

on Ca-rich or Ca-poor soil did not differ in shell breadth ($F_{1,120} = 0.0001$, $p = 0.99$).

All snails attained adult size within four weeks. Fully grown snails from the two populations differed in adult size (shell breadth: Gurnigel: 17.3 ± 0.1 mm; Zastler: 19.4 ± 0.1 mm; $F_{1,121} = 124.59$, $p < 0.0001$). In both populations, adult shell size was not affected by the type of soil on which the snails were kept ($F_{1,121} = 1.70$, $p = 0.20$). Relative shell growth differed among snails from the two populations (Gurnigel: 0.155 ± 0.008 ; Zastler: 0.084 ± 0.006 ; $F_{1,120} = 53.92$, $p < 0.0001$) and was slightly, but not significantly higher in snails kept on Ca-rich soil than in snails kept on Ca-poor soil ($F_{1,120} = 2.65$, $p = 0.106$).

Mating Propensity

Copulations were observed in 75 (34.9%) out of 215 trials. Snails from the Gurnigel population showed a slightly but not significantly higher mating propensity (40.4%; 40 matings out of 99 trials) than those from the Zastler population (30.2%; 35 matings out of 116 trials; $\chi^2 = 2.46$, $df = 1$, $p = 0.13$). However, the type of soil affected the mating propensity. Independent of origin, snails kept on Ca-poor soil showed a higher mating propensity than snails kept on Ca-rich soil (43.9% vs. 27.4%; $\chi^2 = 6.41$, $df = 1$, $p = 0.015$, data from both populations combined). In the Zastler population, a larger proportion of snails kept on Ca-poor soil copu-

lated compared with snails kept on Ca-rich soil (39.6% vs. 22.6%; $\chi^2 = 3.92$, $df = 1$, $p = 0.048$). Similarly, in the Gurnigel population, there was a tendency that more snails kept on Ca-poor soil copulated than snails kept on Ca-rich soil (50.0% vs. 33.3%; $\chi^2 = 2.79$, $df = 1$, $p = 0.08$). The time from initiation of courtship to copulation ranged from 6.5 to 24.5 h (mean: 12.3 h, $n = 69$) and was neither influenced by the type of soil ($F_{1,65} = 0.04$, $p = 0.84$) nor did it differ between the two populations ($F_{1,65} = 0.12$, $p = 0.73$).

Female Reproductive Output

Snails deposited their first egg batch 10 to 39 days after copulation (mean: 13.1 days, $n = 69$). The time elapsed between copulation and first oviposition was neither influenced by the type of soil ($F_{1,65} = 0.19$, $p = 0.67$) nor did it differ between populations ($F_{1,65} = 0.01$, $p = 0.99$).

Snails from either population did not differ in number of egg batches produced (Table 2). However, the number of egg batches produced was affected by soil type (Table 3). Surprisingly, snails kept on Ca-poor soil deposited more batches than those maintained on Ca-rich soil (Table 2). Furthermore, the number of egg batches laid was influenced by shell size (Table 3). Similarly, the total number of eggs produced by each snail was affected by soil type (Table 3). Snails kept on Ca-poor soil produced more eggs than snails kept of Ca-rich soil (Table 2). The total number of eggs produced was not

TABLE 2. Female and male reproductive traits of *A. arbustorum* from two populations (Gurnigel and Zastler). Snails were kept both on Ca-rich soil (original substratum in Gurnigel) and Ca-poor soil (original substratum in Zastler). Mean values ± 1 S.E. with sample size in parentheses are presented. Results of ANCOVAs are shown (for details of statistical analyses, see Table 3).

Trait	Gurnigel population		Zastler population		Effect of		
	Ca-rich	Ca-poor	Ca-rich	Ca-poor	shell size	population	soil type
Number of egg batches	3.7 ± 0.5 (15)	4.0 ± 0.3 (21)	3.8 ± 0.4 (14)	4.5 ± 0.3 (19)	*	ns	*
Total number of eggs	116 ± 14 (15)	129 ± 12 (21)	139 ± 15 (14)	167 ± 11 (19)	ns	ns	*
Egg mass (mg)	2.45 ± 0.09 (12)	2.18 ± 0.04 (12)	2.34 ± 0.14 (12)	2.44 ± 0.10 (12)	*	**	ns
Number of sperm delivered ($\times 10^3$)	2194 ± 340 (15)	2035 ± 276 (20)	2705 ± 450 (13)	2049 ± 230 (19)	ns	ns	ns
Spermatophore size, (volume of sperm container, mm^3)	1.95 ± 0.21 (15)	1.75 ± 0.15 (20)	2.68 ± 0.28 (14)	2.21 ± 0.19 (19)	*	ns	ns

* $p < 0.05$, ** $p < 0.01$, ns = not significant

affected by shell size (Table 3). In contrast, egg dry mass differed between populations and was significantly related to shell size, but was not affected by soil type (Table 3). Furthermore, the analysis of covariance had a significant interaction term (shell size x population), indicating differing slopes between populations (Table 3). In snails from the Zastler population, egg dry mass was positively related to the shell size of the mother snail ($R^2 = 0.52$, $n = 8$, $p = 0.0424$), whereas in the Gurnigel population no significant relationship was found ($R^2 = 0.08$, $n = 8$, $p = 0.49$).

Hatching success of eggs differed slightly, but not significantly between populations (Gurnigel: $82.3 \pm 2.6\%$, Zastler: $75.3 \pm 3.4\%$; $F_{1,65} = 3.62$, $p = 0.0617$), but was not affected by soil type ($F_{1,65} = 2.46$, $p = 0.12$).

Female reproductive output, represented by the dry mass of all eggs produced by an

individual snail, ranged from 21 to 614 mg (mean: 327 mg, $n = 69$). Female reproductive output was affected by soil type (Ca-rich soil: 299 ± 25 mg; Ca-poor soil: 347 ± 21 mg; $F_{1,61} = 5.14$, $p = 0.0270$), but did not differ between the populations ($F_{1,61} = 0.25$, $p = 0.62$) and was not influenced by snail size ($F_{1,61} = 0.17$, $p = 0.68$). There was, however, a significant interaction term (shell size x soil type), indicating that shell size influenced female reproductive output on different soils in a different way ($F_{1,61} = 4.92$, $p = 0.0302$). The dry weight of the albumen gland, another measure of female reproductive output, ranged from 8.7 to 59.4 mg (mean: 29.5 mg, $n = 66$). As the dry mass of all eggs, the mass of the albumen gland was affected by soil type ($F_{1,58} = 4.45$, $p = 0.0392$), but no effects of population and shell size were found ($F_{1,58} = 0.87$, $p = 0.36$ and $F_{1,58} = 0.21$, $p = 0.65$).

TABLE 3. Analyses of covariance (ANCOVA) of the relationship between reproductive characters and shell size (volume, log-tranformed) of *A. arbustorum*. Snails from two populations were kept each on two soils (only significant interactions are shown).

Dependent variable	Independent variable, Effects	df	Type III SS	F	p
Number of egg batches (log)	Shell size	1	0.231	6.44	0.0137
	Population	1	0.035	0.97	0.33
	Soil type	1	0.182	5.07	0.0279
	Shell size x soil type	1	0.167	4.66	0.0347
	Error	61			
Total number of eggs	Shell size	1	518.622	0.18	0.68
	Population	1	635.611	0.22	0.64
	Soil type	1	15101.587	5.14	0.0269
	Shell size x soil type	1	14582.744	4.97	0.0295
	Error	61			
Egg mass (log)	Shell size	1	0.018	10.27	0.0125
	Population	1	0.023	13.53	0.0062
	Soil type	1	0.001	0.58	0.47
	Shell size x population	1	0.017	10.02	0.0133
	Error	8			
Number of sperm delivered (log)	Shell size	1	0.173	1.64	0.21
	Population	1	0.051	0.48	0.49
	Soil type	1	0.00003	0.003	0.99
	Error	59			
Spermatophore size, (volume of sperm container, log)	Shell size	1	0.077	5.53	0.0220
	Population	1	0.0001	0.01	0.92
	Soil type	1	0.002	0.13	0.72
	Error	60			

Male Reproductive Output

Spermatophore size, indicated by the volume of sperm container, was neither influenced by soil type nor by population (Table 3). However, spermatophore size was influenced by shell size (Table 3); it was positively correlated with shell volume ($r = 0.42$, $n = 68$, $p = 0.0003$). The number of sperm delivered in a spermatophore ranged from 192,000 to 6,026,000 (mean: 2,204,600, $n = 67$). Sperm number was neither affected by soil type, nor by the origin of the snails (population) and shell size (Tables 2, 3).

The male reproductive output, expressed as dry mass of both spermatozoa and spermatophore, ranged from 0.31 to 1.83 mg (mean: 0.96 mg, $n = 68$). Male reproductive output was affected by shell size ($F_{1,60} = 5.01$, $p = 0.0289$), but not by soil type and population ($F_{1,60} = 0.05$, $p = 0.83$ and $F_{1,60} = 0.11$, $p = 0.74$).

The relative allocation to the male reproductive function, expressed as percentage of the total dry mass devoted to the male function, ranged from 0.1 to 3.2% (mean = 0.4%, $n = 68$). The remaining 96.8 to 99.9% of the dry mass were allocated to the female reproductive output. The relative allocation to the male reproductive function was neither influenced by soil type, population nor by shell size (in all cases $p > 0.20$).

DISCUSSION

The present study showed that the type of soil can affect the mating propensity and female reproductive output in a simultaneous hermaphrodite land snail. Mating propensity and other life-history traits including adult shell size, shell growth, and egg size were also influenced by the snails' origin, and still others (egg size, number of egg batches produced and spermatophore size) were affected by shell size. Effects of soil type on reproductive traits have so far received little attention in terrestrial gastropods. The breeding behaviour of *Cernuella virgata* (da Costa, 1778) was affected both by soil type and soil moisture content, whereas the total number of eggs laid and the tendency to lay the first egg were influenced by soil type (Carne-Cavagnaro et al., 2006). Juveniles of *Cornu aspersum* (Müller, 1774) from different sites exhibited differential growth rates and Ca-allocation strategies (Beeby & Richmond, 2007). Land snails may be able to assess the Ca concentration in their food plants and in the

soil (Chevalier et al., 2003) and may actively balance their diet to optimise the Ca intake (Iglesias & Castillejo, 1999). Soil constitutes a key component of the snails' diet in natural populations. In *C. aspersum*, hatchlings which fed on soil outgrew other siblings, indicating the importance of certain soil characteristics and of exchangeable Ca (Gomot et al., 1989).

In a laboratory experiment, the Ca concentration did not affect shell growth and adult size in subadult individuals of *A. arbustorum* reared on agar-based diets with different levels of Ca (Wacker & Baur, 2004). Snails reared on intermediate- and low-calcium diets increased their consumption rates, but despite compensatory feeding, these snails were unable to take up the amount of Ca required for metabolism and shell growth and thus had a higher mortality.

The Ca-provision to the eggs represents a major cost to the parent. Among gastropods, a variety of strategies are used to ensure sufficient Ca to the eggs (Tompa, 1980; Baur, 1994a). Ca is used for the calcification of the embryonic shell and for the deposition of Ca reserves which differentiate during the embryonic life (Fournié & Chétail, 1984). The Ca requirements for embryonic life explain the considerable amount of Ca that the adult gastropod loses during the oviposition period. For example, *Anguispira alternata* (Say, 1816) mobilizes 10–25 mg Ca for one egg batch in less than a day (Tompa, 1975).

In several species of terrestrial gastropods, a significant amount of Ca is embedded in the egg shell. *Arianta arbustorum* has partly calcified eggs with discrete crystals of calcium carbonate in a jelly matrix. Compared with species that produce similar eggs, the Ca concentration in *A. arbustorum* eggs may range from 5 to 8% of their dry mass (cf. Tompa, 1976). Thus, snails kept on Ca-poor soil may have invested 17–28 mg Ca into the eggs produced in the course of the experiment, those kept on Ca-rich soil 15–24 mg Ca. The Ca-content of lettuce varies between 300 and 500 mg/kg (Chevalier et al., 2003). Individual *A. arbustorum*, kept under similar experimental conditions as in the present study, consumed a leaf area of 32–50 cm² lettuce per week, which corresponds to a Ca-uptake of 0.26 to 0.68 mg per week (Locher & Baur, 2002). The assimilation efficiency of consumed lettuce averages 97% in *A. arbustorum* (dry mass; Abdel-Rehim, 1987). Considering the entire experimental period of 88 days, this resulted in a Ca-uptake of 3.3–8.5 mg, indicating that a considerable amount of Ca invested in egg

production was actually obtained from the lettuce consumed. Thus, snails kept on Ca-poor soil could partly compensate the Ca-deficiency in the soil by consuming more lettuce. It should be noted, however, that the experimental conditions in our study did not represent the natural situation, because plants growing on Ca-poor soils usually have a low Ca-content. The same might be true for decaying plant material and leaf litter on Ca-poor soil.

Scarcity of Ca may result in thinner and more brittle shells (Voelker, 1959), rendering the snails less fit to protect the soft body properly against desiccation, physical damage and invertebrate predators. *Arianta arbustorum* shows a tolerance to acidic soils with low Ca availability (Kerney & Cameron, 1979). In the wild, however, adult *A. arbustorum* with more robust shells were found at the Ca-rich site at Gurnigel than at the Ca-poor site at Zastler (B. Baur, unpubl. data).

Because snails require substantial amounts of Ca for reproduction (Crowell, 1973; Wäreborn, 1979), growth (Gomot et al., 1989; Ireland, 1991), shell production, and metabolism (Fournié & Chétail, 1984), Ca availability is a key determinant of habitat quality in molluscs. In fact, greater abundance, species richness, and biomass of snails are generally observed on soils rich in Ca compared with soils poor in Ca (Boycott, 1934; Wäreborn, 1992; Hotopp, 2002; Ondina et al., 2004). Ca addition to mitigate effects of acid deposition resulted in a significant increase in snail abundance (Gärdenfors, 1992; Skeldon et al., 2007), whereas slugs did not show a similar pattern. However, processes other than acid deposition also influence Ca cycling and availability in soils. For example, wood succession resulting in a shift in the dominant tree species can change the foliar Ca concentration.

In suboptimal habitats where conditions are outside the range to which most snail individuals are adapted, some cost, expressed either as a reduction in growth, survival, or reproduction, may occur. However, apart from Ca availability, other soil parameters, such as soil physical properties (soil texture, moisture retention and chemistry) and availabilities of nutrients such as aluminium, nitrogen and magnesium, may influence the reproductive output of *A. arbustorum*. Soil type seems to be a key factor in the reproductive biology of *A. arbustorum* and most probably in other land snail species. However, it remains unclear which soil characteristics are important.

In our study, the total number of eggs produced was larger in snails kept on Ca-poor soil than in individuals maintained on Ca-rich soil. There are different possible explanations for this surprising finding: (1) the Ca-poor soil used in the experiment still contained enough Ca to allow egg production; (2) the snails ingested Ca from the lettuce offered and with compensatory feeding they partly counter-balanced the Ca deficiency; (3) the Ca-rich soil could contain minerals or (unknown) substances that disturbed reproduction in *A. arbustorum*; (4) the Ca-poor soil had other essential constituents for successful reproduction that were in short supply in the Ca-rich soil; (5) snails living under environmental stressing conditions (i.e., on Ca-poor soils) put more resources into reproduction in their first breeding but have a shorter lifespan than snails in more favourable conditions. The range of Ca content in different soil types is much wider in nature than in the two soils used in this experiment. Our Ca-poor soil still contained a low level of exchangeable Ca (Table 1). The hypothesis of compensatory feeding cannot be tested because the amount of lettuce consumed by each snail was not recorded. However, the albumen gland of snails kept on Ca-poor soil was larger than that of snails maintained on Ca-rich soil. This indicates that the former group ingested more food. In stylommatophoran gastropods, the albumen gland synthesizes the perivitelline fluid to be added to eggs for provisioning the developing embryo (Gomez, 2001). The size of this gland determines the maximum number of eggs that can be produced at any one time (Tompa, 1984). Most probably, snails kept on Ca-rich soil consumed for some unknown reasons less lettuce, which resulted both in a decreased mating propensity and reproductive output. Hatching success of eggs produced by snails kept on either soil type did not differ, suggesting that there was no difference in egg quality between the two treatment groups. At high concentrations, zinc, copper, lead and cadmium all negatively affect reproduction in *C. aspersum* (Laskowski & Hopkin, 1996). At low concentrations, however, no effect of these metals was recorded (Gomot, 1997). In contrast, other soil constituents may positively affect snail reproduction. For example, the Ca-poor soil contained three times more magnesium than the Ca-rich soil (Table 1). Unfortunately, besides Ca relatively little is known on the beneficial value of various soil constituents for land snail reproduction (Speiser, 2001). There may also

be a trade-off between reproductive output and survival. Snails living in environmentally stressing conditions may allocate more resources into reproduction in the first breeding season but may die earlier than those living in more favourable conditions. This hypothesis could be tested by maintaining snails over two or more years under the experimental conditions of the present study. We assume that a combination of different factors contributed to the unexpected result.

The present study examined to our knowledge for the first time soil-type related effects on male reproductive output. However, neither the number of sperm delivered nor spermatophore size differed between the two snail groups kept on different soils. Of the total reproductive output (expressed as dry mass), only 0.1–3.2% were devoted to the male function in form of sperm and spermatophore, while the remaining 96.8–99.9% were allocated to the female function in form of eggs. These values are very similar to previous estimates of male and female reproductive allocation in *A. arbustorum* (Locher & Baur, 2000, 2002).

In our study, several life-history traits were affected by the origin of the snails and/or their adult size. Size-related fecundity has been found in a variety of gastropods as well as in other invertebrates (Baur, 1998). In many land snail species, both clutch size and egg size are positively correlated with shell size (Heller, 2001). In the present study, the number of egg batches deposited, egg mass and spermatophore size were related to adult size in *A. arbustorum*.

In land snails, limited dispersal capacity and restricted habitat requirements may enhance the process of adaptation to local conditions. Genetic differentiation between populations of terrestrial gastropods has been demonstrated in several species (e.g., Jones et al., 1977; Clarke et al., 1978; Fearnley, 1996; Beeby & Richmond, 2001, 2007). The adult size of *A. arbustorum* decreases with increasing altitude in the Alps, whereas shell colour varies with the type of habitat (Burla & Stahel, 1983; Baur, 1984; Gosteli & Burla, 1993). Snails from different populations exhibit differences in resting site preference and geotactic response (Baur, 1986; Baur & Gosteli, 1986), as well as in such reproductive characters as clutch size and egg size (Baur & Raboud, 1988). Snails from the two populations examined in the present study differed in adult size, relative shell growth, mating propensity and egg size.

In conclusion, the present study showed that soil type can affect reproductive traits in *A. arbustorum*. However, some reproductive traits were also influenced by the origin of the snails and by shell size emphasizing the importance of proper design and replication of life-history studies in gastropods.

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